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Honeybee odometry and scent guidance

Tony Vladusich^{1,*}, Jan M. Hemmi² and Jochen Zeil²

¹*Laboratory of Experimental Ophthalmology and NeuroImaging Centre, School of Behavioural and Cognitive Neurosciences, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands and*

²*Centre for Visual Science, Research School of Biological Sciences, Australian National University, Canberra, ACT 2600, Australia*

*Author for correspondence (e-mail: t.vladusich@med.umcg.nl)

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Summary

We report on a striking asymmetry in search behaviour observed in honeybees trained to forage alternately at one of two feeder sites in a narrow tunnel. Bees were trained by periodically switching the position of a sucrose reward between relatively short and long distances in the tunnel. Search behaviour was examined in the training tunnel itself and in a fresh tunnel devoid of scent cues deposited by bees during training. Bees tested in the fresh tunnel exhibited a bias towards the shorter site, while bees tested in the training tunnel searched closer to the longer site. In additional experiments, we manipulated the position of

scent cues, relative to the training location, in the testing tunnel. Bees generally searched at the site to which they were trained rather than at the position of the scent. Our data argue strongly against the hypothesis that bees rely exclusively on deposited scent to accurately localise a food source in natural foraging environments. We instead conclude that odometry and scent guidance contribute to honeybee food search in a manner reflecting the significance and relative reliability of sensory information.

Key words: navigation, honeybee, odometry, scent, *Apis mellifera*.

Introduction

Honeybees use a combination of sensory cues to guide navigation (von Frisch, 1993). These include both long-range cues, such as visual odometry (distance flown) and compass direction, and short-range cues, such as olfactory and visual-landmark cues. An outstanding problem concerns how bees integrate these sensory cues to guide their return to various places in the environment, such as the location of a food source.

In the present study, we modify the experimental protocols introduced by Srinivasan and coworkers (Srinivasan et al., 1996; Srinivasan et al., 1997; Srinivasan et al., 1998) to investigate visual odometry. We ask, where do bees search when trained along a path upon which the food source is shifted periodically between a distance near the entrance of a short, visually textured tunnel and a second distance further away from the entrance? The rationale is that the two training distances provide bees with ambiguous information about food location. Since the food could be either at the short distance or the long distance, bees must make a choice at some point along the route to begin searching at one or other site. Alternatively, bees might average the two training distances (Menzel et al., 1998) and so search in the region between the two sites. It is also possible that bees exhibit a type of recency effect in memory, whereby they search at the most recently rewarded site. One aim of the experimental protocol is therefore to

examine how bees assign relative significance to conflicting odometric cues and, in so doing, to reveal aspects of the way in which bees encode and memorise information about the environment (Collett et al., 1993; Collett and Collett, 2000; Collett et al., 2002; De Marco and Menzel, 2005; Giurfa and Capaldi, 1999; Menzel and Giurfa, 2001; Menzel et al., 1998; Menzel et al., 2005; Wehner and Menzel, 1990; Wehner et al., 1990).

A second aim of the present study is to examine how odometry-based search performance might be modified by the presence of scent cues deposited by bees upon visiting the food source (see Wolf and Wehner, 2000; Wolf and Wehner, 2005 concerning use of scent cues by desert ants). Few previous studies have sought to characterise the role of scent guidance in scaled-down honeybee foraging environments (Fry and Wehner, 2002). That bees release scent from the Nasanov gland at a feeder site, possibly to inform hive mates of the location of a food source (Wenner, 2002), has been known since the time of von Frisch (von Frisch, 1993). Studies of visual odometry often seek to control for the potentially confounding effects of scent on search behaviour by testing bees in (fresh) tunnels devoid of scent (e.g. Srinivasan et al., 1997). Bees are sometimes reluctant, however, to enter a fresh tunnel, a problem that is sometimes solved by periodically feeding bees at randomly varying locations in the tunnel in which they are to be tested (e.g. Srinivasan et al., 1998). Here,

we characterize the influence of scent cues on search performance in the dual-distance protocol outlined above and in experiments involving training to only one site. We manipulate the presence/absence and location of scent cues in the testing tunnel (see Materials and methods) in order to better understand how honeybees combine learned odometric information with available scent cues to guide food search.

Materials and methods

Location and equipment

All experiments were conducted indoors in the All Weather Bee Flight Facility at the Research School of Biological Sciences at the Australian National University. A single colony of Italian honeybees (*Apis mellifera* L.) was trained to forage at a feeder located within a tunnel lined with a checkerboard pattern made of cloth. Bees flew from the hive to the tunnel, located around 3 m away. The tunnel was 0.2 m high, 0.22 m wide and either 3.4 m or 7.8 m long. The entire set-up was enclosed in a white cloth tent that separated the experimental environment from the rest of the bee house. The roof of the tent was at a height of 1.5 m above the tunnels and was 2 m wide. The sides of the tent extended down below the height of the tunnels, leaving a passage roughly 0.5 m between the outer side of each tunnel and the edge of the cloth. Bees entered and exited the experimental set-up from below the sides of the tent. The role of the tent was to prevent bees from using external landmark cues to locate the feeder. A wooden board, with a portion cut out of it to match the shape of the tunnel entrance, was placed at the entrance to guide bees into the training or testing tunnel. This was done to partially mitigate against the problem of bees not entering a fresh tunnel. The consistent colour and shape of the wooden board tended to facilitate the bees' orientation towards the tunnel entrance.

The side of each tunnel was marked every 0.2 m, thereby enabling quantification of search patterns. The entire tunnel was covered with nylon mesh to prevent bees from entering or exiting at any location other than through the tunnel entrance. The cloth pattern was affixed to the tunnel by means of Velcro™ strips attached to both the cloth and tunnel. In each experiment, approximately 20 bees were marked individually with coloured paint and trained for several hours to locate a food reward. A control experiment ensured that external landmarks did not influence search behaviour. Namely, bees trained in a tunnel lined with axial stripes searched as if they could not locate the position of the food reward (Srinivasan et al., 1996; Srinivasan et al., 1998).

Training

The feeder was a small plastic container (100 ml capacity), with a flat circular-shaped base through which bees could extract small amounts of sucrose solution. The feeder was placed on the floor of the tunnel on top of a small transparent sheet of plastic. The sucrose concentration was 1 mol l⁻¹ at the start of training but was modulated slightly throughout the experiment to keep an approximately constant number of bees

coming to the experiment. For each experiment, the feeder was initially placed at the tunnel entrance and progressively moved to the longer of the two training distances.

Food search

After an initial training session of 8 h, bees were tested in 5 min sessions every 2 h. The brevity of the test sessions kept the bees highly motivated and therefore more likely to enter the testing tunnel. The entire testing protocol ran for at least 2 days, in which bees were exposed to each testing condition at least twice. The order in which conditions were tested was randomised within blocks, each block testing all conditions (once tested, a condition was excluded until all others were tested). Each block was tested at least twice. During tests, the feeder was removed and individual bees flew into the same tunnel in which they were trained. U-turns were recorded visually by the experimenter. A U-turn was classified as an event in which a bee reversed its direction of travel in the tunnel.

Specifics of the dual-distance protocol

Two tunnels were arranged side-by-side and the positions of the training and fresh tunnels were interchanged every 2 h to prevent bees from associating the spatial position of the training tunnel, but not the fresh tunnel, with reward. Bees were trained in blocks (e.g. 1 h) in which feeder position varied between short and long distances. In one block, the feeder was initially positioned at one of the two locations (e.g. short distance for 15 min) and then positioned at the other location (i.e. long distance for 15 min). In another block, the opposite temporal arrangement was implemented. During training, bees were observed carefully to ensure that they visited each feeder site. In most experiments, the number of visits to each site was quantified (see Results). Bees were tested after every second training block, giving rise to two test conditions. One test condition examined the bees' search patterns after they had last been trained to the shorter tunnel distance, while the other condition tested bees after they had last fed at the longer distance. This testing protocol therefore enabled the quantification of any short-term bias toward the location the bee last visited. The test sessions were interleaved (e.g. last trained at short distance, last trained at long distance, last trained at short distance, etc.), such that the location to which bees were last trained was tested every 4 h. In Experiment 1, bees were trained to forage alternately at units 5 and 10 and tested in the training tunnel and in a fresh tunnel, respectively. In Experiment 2, the training and testing protocol was the same, except that bees were trained alternately at units 5 and 15.

Specifics of the scent-position protocol

The scent-position protocol is shown schematically in Fig. 1. The general methods were similar to those described above, except for the following differences. Tunnels were 3.4 m long, lined with a checkerboard cloth that was 3 m long. A mirror was placed at the end of each tunnel in order to generate the illusion that the tunnel was longer than it really was. This was

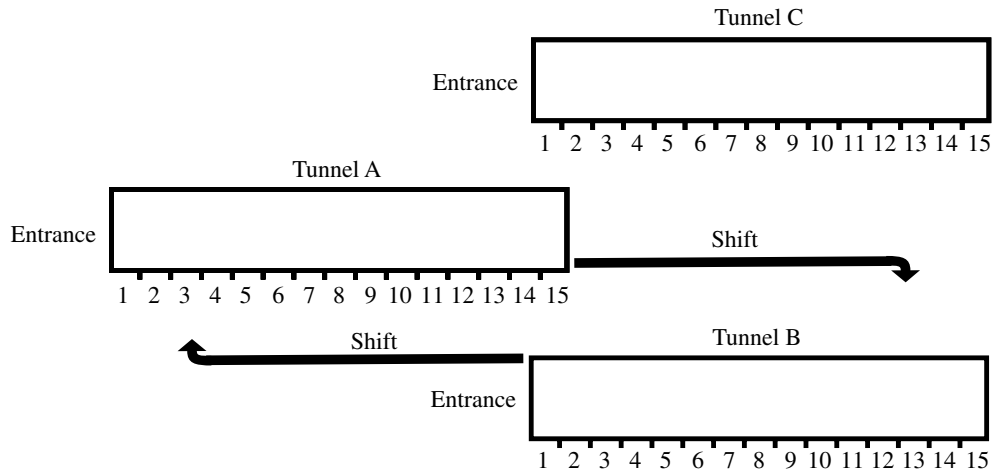


Fig. 1. Schematic illustration of the set-up for scent-position experiments. The set-up and protocol were similar to those used in the dual-distance experiments. The main difference was that, in the scent-position experiments, two groups of bees were separately trained to forage in separate tunnels (Tunnels A and B). The rationale was to train bees to a particular location, thereby allowing bees to learn a specific odometry reading, and then to examine search behaviour in a situation where scent information conflicted with the odometry reading. To this end, bees were tested either (1) in their 'own' training tunnel (e.g. Tunnel A) following a procedure to mimic shifting of the tunnel (dummy-shift condition), (2) in their own tunnel immediately following a training session (no-shift condition), (3) in the tunnel in which the second group of bees were trained at a different position (unit 5/10 tunnel condition) or (4) in a fresh tunnel (Tunnel C) devoid of scent (fresh tunnel condition).

done to mitigate against the possibility of bees using the end of the tunnel as a landmark cue. To manipulate the position of scent cues, two different sets of bees from the same hive were trained simultaneously to different distances in one of two different tunnels (Tunnel A or Tunnel B). That is, in each experiment, bees were individually marked and trained to enter either Tunnel A or Tunnel B (with the feeder position depending on the tunnel). Careful observations were made to ensure that no bee was visiting both tunnels. Any bee found to be visiting both tunnels was killed.

At the beginning of a test session, Tunnels A and B were closed and a feeder placed near the entrance of each tunnel. To test bees in the opposite tunnel to the one in which they were trained, Tunnels A and B were swapped (Fig. 1). A 'dummy-shift' procedure was also undertaken to test whether simply moving a tunnel away from the training location for a few moments, without manipulating the scent pattern other than through increased air flow, was enough to influence the bees' behaviour. Bees were also tested in a 'fresh tunnel' (Tunnel C); that is, a tunnel in which bees had not previously been trained and so was devoid of scent cues. Experiments 3 and 4 were repeated three and two times (respectively), each time with a different group of bees, in order to obtain large enough samples to draw meaningful conclusions.

Data presentation

The U-turns, search distributions and patterns of individual flight paths are presented and analysed in the same fashion as in Vladusich et al. (Vladusich et al., 2005). For each condition, search distributions were calculated on the basis of the first two U-turns (although we recorded four U-turns). The first two U-turns typically provide sufficient information to analyse search

behaviour (Cheng et al., 1999; Vladusich et al., 2005). The search distribution of a group of bees was calculated for each test condition, as follows. For each flight, all tunnel units between the positions of first and second U-turns were assigned values of one. Each of these values was then divided, or weighted, by the total path length between the first and second U-turns (inclusive). These weighted scores were then summed, for each tunnel unit, across all the flights in an experimental condition and divided by the total number of flights. Thus, the total area under the curve representing the search distribution was normalized to one. Due to the normalization with respect to path length (i.e. distance from first to second U-turns), each flight segment contributed the same area to the curve. That is, shorter path lengths (associated with the more accurate searches) contributed the same *bulk* to the search distribution as longer path lengths. Shorter (more accurate) path lengths, however, contributed more to the *height* of the search distribution, because the value associated with each tunnel unit was higher.

All figures also show the position of first and second U-turns normalized to the total number of flights, giving the relative frequency of U-turns across all units. In analysing only the first two U-turns, it is natural to graphically display the flight path-segment between first and second U-turns for each individual flight. This turns out to be a very useful graphical tool, supplementing the histogram representations of U-turns (which do not give information about individual flight paths). Indeed, displaying individual flight paths makes it possible to immediately make the visual link between U-turn position and the search distribution. Thus, each figure displays three types of information: (1) the positions of first and second U-turns plotted as normalized frequency histograms consisting of black and grey bars, respectively, (2) the search

distribution overlaid on the histograms, and (3) the path segments between the first and second U-turns for each flight. The black and grey squares of each path segment indicate positions of first and second U-turns, respectively. For each flight, the line joining the black and grey squares indicates the length of the path segment.

Statistics

Statistical analyses were conducted on the first U-turn data (Kolmogorov–Smirnov test) for the dual-distance experiments (i.e. data not amenable to parametric analysis) and on the means and ranges of first and second U-turns (ANOVA) for the scent-position experiments (range equals the difference between second and first U-turns). These analyses were conducted for each experiment and indicate whether the test tunnel and the most recent training position had an effect on where bees initiated search. We did not attempt to include bee identity in our statistical analyses due to problems in positively identifying bees during the testing sessions (although all tested bees were identified as being marked, it was not visually possible to identify individuals). Analyses were performed using Matlab software, version 6.1 (The MathWorks, Inc., Natick MA, USA).

Results

Dual-distance experiments

Experiment 1: training at units 5 and 10

To ensure that each bee visited both training sites in Experiment 1, the number of visits to each site was recorded

for a period of 1 h. The reward frequencies did not differ between sites ($\chi^2=4.3$, $P\geq 0.1$). Similar data were obtained for the remaining dual-distance experiments.

Fig. 2 illustrates the results for the two test conditions, in which bees were tested in the ‘training tunnel’ condition (Fig. 2A) or the ‘fresh tunnel’ condition (Fig. 2B). Different groups of bees were used in each condition (cf. Experiment 2). In the training tunnel condition, bees initiated search closer to the longer site than bees tested in the fresh tunnel condition. A statistical test conducted on the first U-turn distributions shows a significant overall difference between the two testing regimes (Kolmogorov–Smirnov test, $P<0.001$). Interestingly, bees tested in the training tunnel sometimes initiated and conducted searches just before the training location corresponding to the longer site. On a few occasions, bees also searched in the vicinity of the shorter site, leading to a bimodal search distribution with peaks at around units 4 and unit 9. No such bimodality is evident in the fresh tunnel condition, although bees sometimes initiated search close to the longer site. It is interesting to note, in this context, that on these flights bees often doubled back to perform second U-turns near the shorter training site.

As described in the Materials and methods, bees were trained in blocks in which the feeder position alternated between short and long sites every 15 min. We did not, however, find any difference between the most recently trained locations (i.e. units 5 or 10) on first U-turns in either the training tunnel or fresh tunnel conditions (Kolmogorov–Smirnov test, $P>0.1$). Thus, we found no evidence for a recency effect of training location.

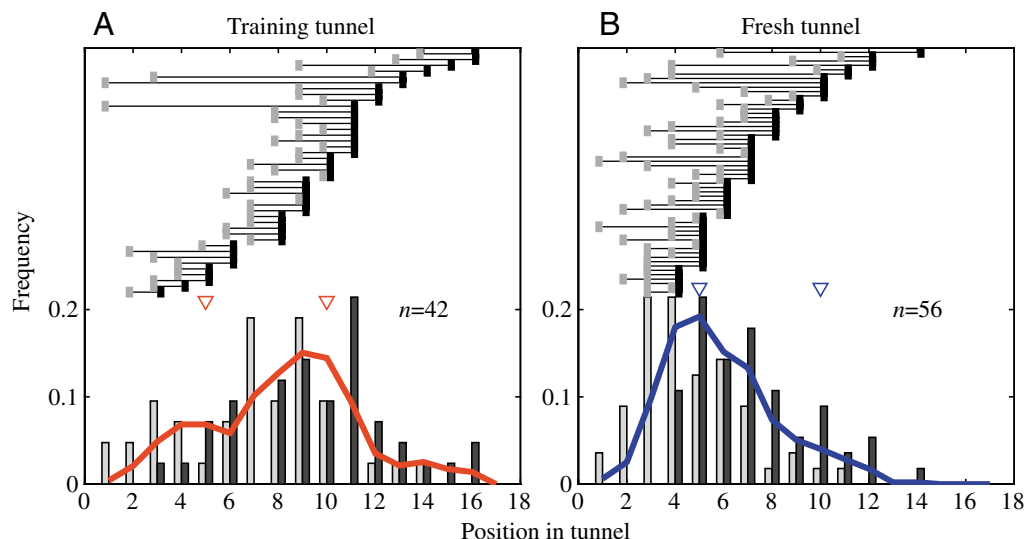


Fig. 2. Results of Experiment 1. Bees trained at units 5 and 10 and then tested in either (A) the training tunnel or (B) a fresh tunnel devoid of scent. Different groups of bees were used in each condition. Bees tested in A were more likely to initiate search (first U-turns; Kolmogorov–Smirnov test, $P<0.001$) closer to the longer site than bees tested in B. Training consisted of blocks in which bees were either rewarded in the sequence short, long, short, long or *vice versa* (the sequence alternated hourly). Data from each condition were collected directly following training to either the short or long site. We found no effect of the most recent training location (i.e. no recency effect of memory) in either condition. Black bars, first U-turns; grey bars, second U-turns; coloured lines, search distributions; inverted triangles, training locations; n , flight number.

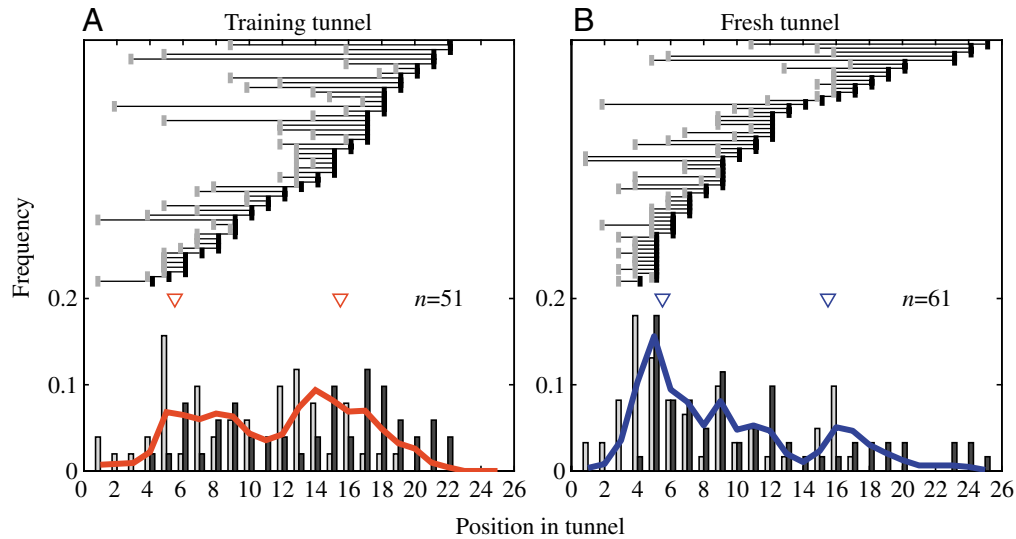


Fig. 3. Results of Experiment 2. A single group of bees was trained at units 5 and 15 using the dual-distance protocol. Similar to the results of Experiment 1, bees tested in the training tunnel (A) were more likely to initiate search (first U-turns; Kolmogorov–Smirnov test, $P < 0.01$) closer to the longer site than bees tested (B) in a fresh tunnel. We found a moderately significant effect of recency for bees tested in the training tunnel condition but not the fresh tunnel condition (see text for details). See Fig. 2 for further details of symbols.

Experiment 2: training at units 5 and 15

Was the search asymmetry described above due to the close proximity of the positions to which bees were trained, or to differences between the two groups of bees used in each experiment? Experiment 2 was conducted in an attempt to answer these questions. The training and testing protocol was the same except that a single set of bees was trained alternately at units 5 and 15.

The pattern of results from Experiment 2 (Fig. 3) closely resembles those described for Experiment 1. The search distribution in the training tunnel condition (Fig. 3A) is biased towards the longer site relative to the fresh tunnel condition (Fig. 3B). The two distributions of first U-turns differ significantly (Kolmogorov–Smirnov test, $P < 0.01$). It should be noted, however, that the individual searches of bees were often distributed between the training sites (see Discussion). We found no recency effect in the fresh tunnel condition (Kolmogorov–Smirnov test, $P \geq 0.1$) and a moderately significant effect of the most recently trained location on first U-turns in the training tunnel condition (Kolmogorov–Smirnov test, $P = 0.014$). Qualitatively, this effect took the form of a slightly increased search bias towards the longer training site.

Scent-position experiments

We hypothesised that the differences in search performance in the dual-distance experiments may relate to putative scent cues deposited by bees during the training protocol. To test this hypothesis, we manipulated the position of putative scent cues in the test scenario independent of the position to which bees were trained (see Materials and methods). Bees were tested (1) in the tunnel to which they were trained, after the tunnel was shifted away and brought back (dummy-shift condition), (2) in the tunnel in which they were trained, immediately after

training (no-shift condition), (3) in the tunnel in which a second group of bees was trained at a different position, and (4) in a fresh tunnel devoid of scent (fresh tunnel condition).

Experiment 3: training at unit 10 alone

Fig. 4 shows the results for the four test conditions in Experiment 3, in which bees were trained at unit 10. The overall impression is that the test tunnel had a large effect on bees' search behaviour. Bees tested in the unit 10 tunnel/dummy-shift condition (Fig. 4A) and the unit 10 tunnel/no-shift condition (Fig. 4B) tended to search at a longer distance than bees tested in the unit 5 tunnel condition (Fig. 4C). The data from the fresh tunnel condition (Fig. 4D) are almost identical to the unit 10 tunnel conditions, indicating that the absence of scent did not affect search behaviour. Interestingly, bees in all conditions except the unit 5 tunnel condition searched just beyond the training position. Since the effect was equally apparent in the fresh tunnel condition, the presence of scent in the training tunnel cannot be the explanation.

By comparison, the search distribution in the unit 5 tunnel condition (Fig. 4C) appears shifted towards the tunnel entrance and there appears to be greater overall spread in this condition. On some flights, bees performed first U-turns slightly beyond the training position but often went on to criss-cross the training site when performing second U-turns. On other flights, bees searched close to unit 5, the position of the putative scent. Bees never searched at this position in the other test conditions.

To quantify the results, an analysis of variance (ANOVA) was conducted on the means and ranges of the first and second U-turns observed for each flight. The ANOVA shows a highly significant overall difference between the means ($F_{3,195} = 5.95$, $P < 0.001$) but not the ranges ($F_{3,195} = 1.07$, $P > 0.1$). Individual

comparisons on the means reveal that the unit 5 tunnel condition differed from the other conditions ($P < 0.01$ for all three comparisons), which themselves did not differ from one another ($P > 0.6$ for all three comparisons), as assessed by a form of paired *t*-test that corrects for unequal variances across conditions (means: unit 10 tunnel/dummy-shift condition=10.72, unit 10 tunnel/no-shift condition=10.83, unit 5 tunnel condition=9.34, fresh tunnel condition=10.9). Taken together, the data in Experiment 3 suggest that scent position in the test tunnel has a significant effect on search behaviour.

Experiment 4: training at unit 5 alone

In contrast to the data above, the results of Experiment 4, in which bees were trained to unit 5, do not exhibit any differences across conditions (Fig. 5). Bees tended to search very accurately at the training position in all four test conditions: the unit 5 tunnel/dummy-shift condition (Fig. 5A), the unit 5 tunnel/no-shift condition (Fig. 5B), the unit 10 tunnel condition (Fig. 5C), and the fresh tunnel condition (Fig. 5D). There was also no evidence of overshoot behaviour in any conditions. Bees in the unit 10 tunnel condition were no more likely to search at unit 10 (i.e. the location of the putative scent) than bees in the two other conditions. An ANOVA conducted on the means ($F_{3,130}=0.27$, $P \geq 0.1$) and ranges ($F_{3,130}=1.21$, $P > 0.1$) of the first two U-turns shows no significant overall differences between the conditions.

In summary, whereas Experiment 3 shows that bees trained at unit 10 then tested in a tunnel with putative scent at unit 5 undershoot the training position, Experiment 4 indicates that bees trained at unit 5 then tested with putative scent at unit 10 do not overshoot the training site.

Discussion

We initiated the dual-distance experiments in a bid to answer questions concerning the nature of visual odometry. How do bees 'decide' where to search when trained to feed at two different sites along a single path in the tunnel environment? In the course of our study, we found that the answer to this question depended on the testing conditions. Specifically, bees tested in a fresh tunnel searched mainly at the shorter site, whereas bees tested in the tunnel in which they were trained searched closer to the longer site. This result did not depend on the distance between the training sites. Since the fresh tunnel was placed in the same location as the training tunnel during testing, external positional cues can be ruled out as a factor.

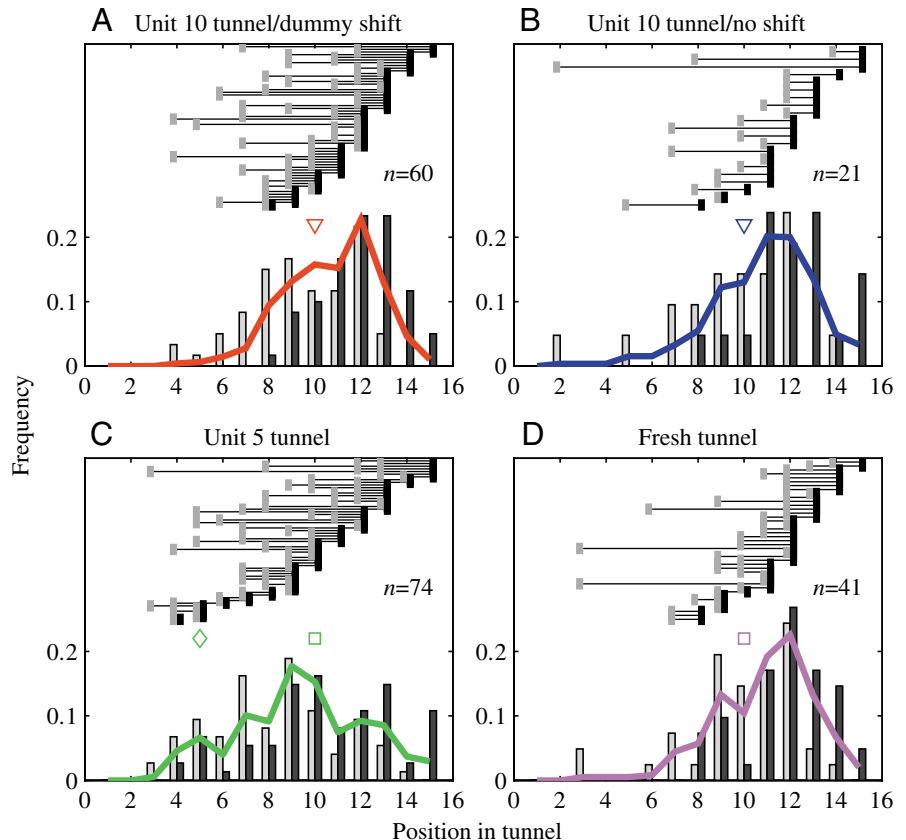


Fig. 4. Results of Experiment 3. Bees trained at unit 10 and then tested in (A) the training tunnel following a 'dummy shift', (B) the training tunnel following no shift, (C) a tunnel in which bees were trained at unit 5 and (D) a fresh tunnel devoid of scent. Bees in C searched at a significantly shorter distance than in A, B and D, indicating that scent 'pulled' searches towards unit 5. Inverted triangles, training location with scent; diamond, scent location; squares, training location without scent. See Fig. 2 for further details.

We hypothesised that scent cues deposited by bees during training may have been responsible for the striking asymmetry in search behaviour exhibited across training and fresh tunnels. Additional experiments, in which two sets of hive mates were trained at different positions in different tunnels, suggest that scent was deposited on the cloth material rather than being *strictly* airborne: no differences were found between the conditions in which bees were tested immediately after training and conditions in which the test tunnel was dummy-shifted, a process that required refreshment of the air in the tunnel. We find only weak evidence to support the hypothesis that bees use scent to accurately localise the position of a memorised foraging locale. Given the controlled, small-scale nature of our experimental environment, it seems unlikely that deposited scent could play a stronger positional role in large-scale foraging environments (Wenner, 2002), where factors such as wind and rain would render scent information less reliable.

Nature and distribution of putative scent

It has long been known that bees mark a feeder site with scent, using the Nasanov gland (von Frisch, 1993), and that scent can be used by both trained bees and naive recruits to

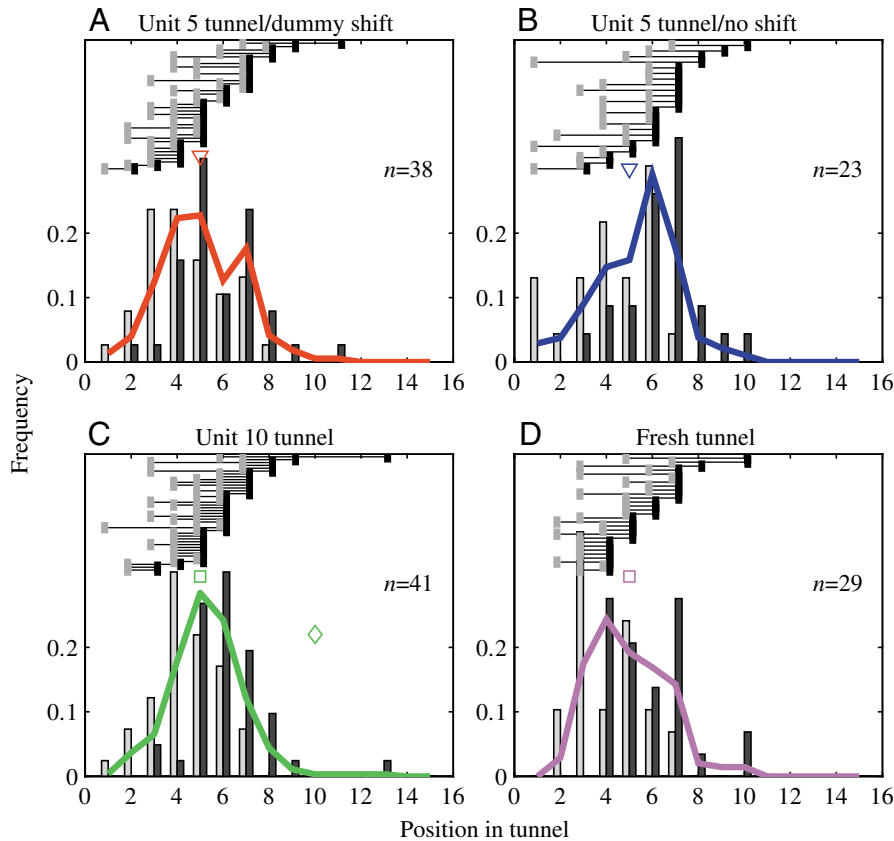


Fig. 5. Results of Experiment 4. Bees trained at unit 5 and then tested in (A) the training tunnel following a 'dummy shift', (B) the training tunnel following no shift, (C) a tunnel in which bees were trained at unit 10, and (D) a fresh tunnel. Mean search position did not differ across conditions, indicating that bees searched in accord with the odometer reading stored in memory rather than the position of the scent. See Fig. 4 for further details of symbols.

help locate the feeder site in outdoor environments. We observed bees 'scenting' the feeder location upon first finding the food source (i.e. raising their abdomens and fanning their wings) but, given the long interval between the start of training and testing (>8 h), it seems unlikely that this was the source of the scent information used by bees in the test situation. A more likely source of scent information comes from bees walking in the vicinity of the feeder throughout the training session. Bees often wandered off beyond the small transparent plastic sheet (10 cm×10 cm) placed at the base of the feeder, potentially distributing scent in the process. Although scent would most likely be deposited on the cloth material near the feeder, the resultant odour plume may have induced behavioural effects at any point in the tunnel. Further investigation, however, is clearly needed to determine the precise nature and distribution of scent in the tunnel environment.

Influence of scent in the dual-distance experiments

Consider the situation confronted by a bee entering the *training* tunnel after having stored in memory the odometer readings associated with the two feeder sites. Upon entering the tunnel and detecting the correct visual and olfactory cues,

the bee continues towards the short site to find the feeder absent. During training, the bee has learned that the absence of the feeder at the shorter site implies its presence at the longer site. The bee therefore continues on towards the longer site (where the feeder is also absent) and initiates search. The precise location and spread of search will depend on many factors, including the distribution of scent in the tunnel and the individual's memories of the two sites. In general, our data indicate that bees initiated and conducted search in the vicinity of the longer site. Bees sometimes initiated search a little closer to the shorter site while, on other occasions, initiated search beyond the longer site and doubled back to perform second U-turns near the shorter site. Such variability in search strategy is of great interest and awaits further study using small numbers of identifiable bees. We suggest here, nonetheless, that our results fail to support the hypothesis that bees learn an average odometer reading from two training sites. This hypothesis can explain neither the rich variety in search behaviour nor the general search asymmetry observed between training and fresh tunnels.

Why did bees search closer to the shorter site when tested in a *fresh* tunnel? A bee entering a fresh tunnel would not smell the expected scent cue. We

observed that bees sometimes either failed to enter the fresh tunnel or aborted flying further shortly after entering the tunnel (units 1 or 2), suggesting that scent did indeed have an influence at the tunnel entrance (i.e. the presence of scent marking the tunnel as 'familiar'). These 'aborted' flights were not included in our sample as they did not represent actual food searches. Those bees that continued flying in the tunnel would arrive at, or slightly beyond, the shorter site. Having failed to smell the scent, these bees executed a fixed search pattern (Wehner and Srinivasan, 1981) rather than continuing towards the longer site. In this sense, scent in the training tunnel probably played a motivational (contextual) role, facilitating the recall and performance of learned flight vectors (Collett et al., 2002; Dale et al., 2005; Fauria et al., 2002) from the shorter to the longer site. That is, the absence of scent in the fresh tunnel discouraged bees from executing the flight vector from the shorter site to the longer site. Recent experiments indicate that bees can associate scent information with specific foraging locales and can recall flight trajectories based on such associations (Reinhard et al., 2004a; Reinhard et al., 2004b). The search behaviour of bees in the dual-distance experiments may therefore reflect the pivotal

contextual role played by scent under natural foraging circumstances.

Influence of scent in the scent-position experiments

Bees in the scent-position experiments were unaffected by the absence of scent at the training site but were moderately affected by the unexpected discovery of scent along the path to the training site. In particular, bees trained at the longer site then tested in a tunnel with scent at (and probably before) the shorter site often searched closer to the shorter site than would otherwise have been the case (Fig. 4). Thus, scent appeared to play more of a positional role than in the dual-distance experiments, facilitating identification of the foraging locale. Scent information did not, however, completely override odometry to the same extent that a nearby visual landmark is known to do (Srinivasan et al., 1997; Vladusich et al., 2005). This diminished efficacy is to be expected given that a nearby visual landmark is an extremely salient cue (Cheng et al., 1987), whereas deposited scent cues, by their very nature, provide diffuse information. It remains likely, therefore, that if one were to train bees to associate a training site with a strong artificial odour (i.e. one deposited by the experimenter), then scent information may override odometry to a greater extent.

The apparent indifference of bees to the absence of scent at the learned site (or its presence beyond the learned site) may reflect the relative reliability of odometry and scent guidance under natural foraging conditions. Environmental factors, such as rain and wind, would ensure that deposited scent provides (at best) intermittent and weak sensory signals. Odometry, by contrast, is far more reliable than scent guidance for the following reasons: (1) variation in measured flight distance can be minimised through learning (Srinivasan et al., 1997), (2) odometric error is predictable and can be compensated by broadening search (Cheng et al., 1999; Srinivasan et al., 1998), (3) the visual inputs to odometry are robust to variations in contrast and spatial frequency (Si et al., 2003) and (4) the visual environment along a foraging path is relatively unaffected by weather conditions, thereby providing a stable information source.

Context-sensitive use of scent information

Bees used scent information in the scent-position experiments and dual-distance experiments in different ways. In the former case, scent played primarily a positional role, shifting search towards the scent locale and away from the training site. In the latter case, scent provided the motivation to recall and perform flight vectors from the shorter training site to the longer site. Such behavioural differences are likely to reflect the flexible manner in which bees assign significance to sensory information in different contexts (Collett et al., 2002; Dale et al., 2005; Fauria et al., 2002; Vladusich et al., 2005).

We suggest that future odometry/scent experiments may fruitfully probe such context sensitivity by combining aspects of the dual-distance and scent-position designs. In particular, one might train bees in the dual-distance paradigm, then test them in

tunnels in which groups of bees have been trained at either the short or long distance. Bees would therefore have odometric information consistent with either site and scent information consistent with only one site. We predict that bees would be strongly biased towards searching near the scented site, since, in this situation, scent provides meaningful and reliable information to disambiguate between the memorised sites.

Summary and conclusions

We find strong evidence that scent information influences honeybee search behaviour in a scaled-down foraging environment. Our results suggest, however, that bees would be unlikely to rely solely on deposited scent cues to locate food sources in the 'blooming buzzing confusion' (James, 1979) of the honeybee foraging environment. Instead, bees appear to combine odometry and scent information in a context-sensitive and flexible manner to form 'intelligent decisions' about the probability of finding food in a particular place (see also Vladusich et al., 2005).

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